STUDIES RELATING TO FERTILITY IN ALFALFA

(*Medicago sativa* L.)

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INTRODUCTION

The high degree of variability in the amount of seed set by alfalfa plants is well known. As alfalfa is one of the more important forage crops in many sections of the world, research workers have, for the past thirty or forty years, been interested in the problem of seed-setting. Plant differences in fertility have been demonstrated by various workers, amongst these Bolton and Fryer (6). It has been realized for a considerable time that climatic and soil conditions affect seed-setting, but very little investigational work has been conducted under controlled conditions. Studies begun in 1936, dealing with some few of the physiological factors which might have a bearing on seed-setting, are here reported.

The report is presented in two sections, since two distinct aspects of fertility in alfalfa were studied.

GENERAL MATERIALS AND METHODS

Studies were carried out on individual plants, the complete list of which is given in Table I. The table includes information regarding two of the important morphological characters of the plants, as well as the varietal origin and fertility rating. All of these plants were collected and classified by Bolton (5).

With one exception, the plants are considered to be representatives of the species Medicago sativa L. Plant S_1.28.3 (9-11), which could be classed as M. media Pers., has yellow flowers, pods which are slightly coiled, and extremely erect stems.

In all cases, except where special mention is made, the plant material used was from clones grown in a large isolation screenhouse.

The statistical analyses were carried out according to the methods outlined by Fisher (21) and Snedecor (29). The inverse-sine transformation was applied to the percentage data following suggestions given by Cochran (16) and Clark and Leonard (14).

TABLE I

List of plant material used

Plant No.	Varietal origin	Flower color	Growth habit	Fertility classification
I.28.18 (14-38)*	Grimm, Disco	medium purple	erect	fertile
S_1.31.1 (23-4)	Grimm, Disco	light purple	erect	fertile
S_2.32.26 (33-4)	Grimm, Disco	light purple	erect	fertile
S_2.32.26 (34-5)	Grimm, Disco	light purple	erect	fertile
S_2.32.29 (40-10)	Grimm, Lyman's	light purple	erect	fertile
I.31.9 (21-23)	Grimm	light purple	erect	fertile
I.31.9 (21-35)	Grimm	light purple	erect	fertile
S_2.32.7 (10-34)	Grimm, Kirk's	light purple	erect	fertile
S_1.32.32 (47-5)	Cossack	bluish	erect	fertile
S_1.28.3 (9-11)	Grimm, Grafton's	yellow	erect	sterile
S_3.33.9 (6-33)	Grimm, Lyman's	light purple	decumbent	sterile
S_3.33.3 (4-5)	Ontario Variegated	dark purple	erect	sterile

* This plant is the same as I.28.18 (8-28), and designated as 8-28f by Bolton and Fryer (6).

Table 1

Sales of new passenger cars

Year	Total	Domestic	Imports
1970	8,387,000	6,546,000	1,841,000
1971	8,823,000	6,713,000	2,110,000
1972	9,294,000	7,119,000	2,175,000
1973	9,167,000	6,985,000	2,182,000
1974	7,173,000	5,390,000	1,783,000
1975	7,056,000	5,231,000	1,825,000
1976	8,542,000	6,648,000	1,894,000
1977	9,147,000	7,054,000	2,093,000
1978	9,174,000	6,942,000	2,232,000
1979	8,473,000	6,581,000	1,892,000
1980	6,581,000	4,320,000	2,261,000
1981	6,255,000	4,208,000	2,047,000
1982	5,759,000	3,842,000	1,917,000
1983	6,712,000	4,567,000	2,145,000
1984	7,627,000	5,267,000	2,360,000
1985	8,185,000	5,812,000	2,373,000
1986	8,214,000	5,815,000	2,399,000
1987	7,893,000	5,454,000	2,439,000
1988	8,098,000	5,553,000	2,545,000
1989	7,760,000	5,127,000	2,633,000
1990	6,838,000	4,218,000	2,620,000
1991	6,280,000	3,885,000	2,395,000
1992	6,709,000	4,197,000	2,512,000
1993	7,409,000	4,715,000	2,694,000
1994	8,151,000	5,238,000	2,913,000
1995	8,066,000	5,127,000	2,939,000
1996	8,135,000	5,128,000	3,007,000
1997	8,095,000	5,044,000	3,051,000
1998	8,274,000	5,128,000	3,146,000
1999	8,660,000	5,324,000	3,336,000
2000	8,822,000	5,362,000	3,460,000
2001	8,541,000	5,125,000	3,416,000
2002	8,423,000	5,000,000	3,423,000
2003	8,423,000	4,970,000	3,453,000
2004	8,423,000	4,940,000	3,483,000
2005	8,423,000	4,910,000	3,513,000
2006	8,423,000	4,880,000	3,543,000
2007	8,423,000	4,850,000	3,573,000
2008	8,423,000	4,820,000	3,603,000
2009	8,423,000	4,790,000	3,633,000
2010	8,423,000	4,760,000	3,663,000
2011	8,423,000	4,730,000	3,693,000
2012	8,423,000	4,700,000	3,723,000
2013	8,423,000	4,670,000	3,753,000
2014	8,423,000	4,640,000	3,783,000
2015	8,423,000	4,610,000	3,813,000
2016	8,423,000	4,580,000	3,843,000
2017	8,423,000	4,550,000	3,873,000
2018	8,423,000	4,520,000	3,903,000
2019	8,423,000	4,490,000	3,933,000
2020	8,423,000	4,460,000	3,963,000
2021	8,423,000	4,430,000	3,993,000
2022	8,423,000	4,400,000	4,023,000
2023	8,423,000	4,370,000	4,053,000
2024	8,423,000	4,340,000	4,083,000
2025	8,423,000	4,310,000	4,113,000
2026	8,423,000	4,280,000	4,143,000
2027	8,423,000	4,250,000	4,173,000
2028	8,423,000	4,220,000	4,203,000
2029	8,423,000	4,190,000	4,233,000
2030	8,423,000	4,160,000	4,263,000
2031	8,423,000	4,130,000	4,293,000
2032	8,423,000	4,100,000	4,323,000
2033	8,423,000	4,070,000	4,353,000
2034	8,423,000	4,040,000	4,383,000
2035	8,423,000	4,010,000	4,413,000
2036	8,423,000	3,980,000	4,443,000
2037	8,423,000	3,950,000	4,473,000
2038	8,423,000	3,920,000	4,503,000
2039	8,423,000	3,890,000	4,533,000
2040	8,423,000	3,860,000	4,563,000
2041	8,423,000	3,830,000	4,593,000
2042	8,423,000	3,800,000	4,623,000
2043	8,423,000	3,770,000	4,653,000
2044	8,423,000	3,740,000	4,683,000
2045	8,423,000	3,710,000	4,713,000
2046	8,423,000	3,680,000	4,743,000
2047	8,423,000	3,650,000	4,773,000
2048	8,423,000	3,620,000	4,803,000
2049	8,423,000	3,590,000	4,833,000
2050	8,423,000	3,560,000	4,863,000

PART I

PHYSIOLOGICAL STUDIES OF ALFALFA POLLEN

A. Pollen Viability as Affected by Seasonal Age of the Plant

The seasonal variability in pod-setting of the alfalfa plant is quite marked, and has been reported by numerous workers. That pollen viability might be involved was thought possible, and in 1936 an experiment was conducted to determine whether the pollen viability changed with an advance in the seasonal age of the plant.

Literature Review.

Working with three species of the genus Crepis, Poole (26) made daily counts of the good and bad pollen produced by plants from the beginning to the end of the flowering period. From these counts he came to the following conclusions:

> "Fluctuation in the percentages of good and bad pollen in pure species is probably not influenced by external factors but by the physiological adjustments made to flowering and senescence.

> "The plotted curve of good pollen grain percentages substantiates this view, indicating further that the daily fluctuation is inconsiderable in a given plant once the adjustments are made."

Methods.

Percentage pollen germination on an artificial medium was taken as a measure of pollen viability. The germination counts were made using the same method as used by Bolton (5).

The medium consisted of 1½ grams of agar and 12 grams of cane sugar in 100 cc. of water. The agar-sugar solution was poured into Syracuse dishes and used as soon as cooled. Pollen was spread over the medium by artificially tripping several flowers a few inches above the surface, two plates being prepared for each plant. The plates were covered, and the pollen allowed to germinate for two hours at room temperature (20-23°C.), after which they were placed in a refrigerator at approximately 0°C. until such time as the counts could be made.

Counts were taken of 100 microscopic fields for each plate, using the 16 mm. objective of a Spencer binocular microscope in combination with 10x eyepieces. To facilitate the counting, the plates were flooded

with a dilute solution of methylene blue chloride. A pollen grain was considered to have germinated if the pollen-tube was longer than the diameter of the grain itself.

The same procedure was followed throughout the flowering season at 14-day intervals, the pollen being spread on the agar-sugar medium at about the same time of day on each occasion.

Experimental Results.

The plants used for this experiment were selected for a wide range in pod-setting ability, selection being based on determinations made by Bolton (5). The average percentage of pod-setting for these plants is presented below, the average being for two selfing tests conducted by Bolton (5) in the summer of 1935.

Plant Number	Pod-setting (Percent)
S_3.33.3 (4-5)	0.00
S_1.28.3 (9-11)	11.67
S_2.32.29 (40-10)	22.23
I.31.9 (21-23)	56.37
S_1.32.32 (47-5)	83.35

The results obtained for the pollen viability counts are presented in Table II and shown graphically in Figure 1.

TABLE II

Pollen viability as affected by seasonal age of the plant, expressed as percentage germination on agar-sugar medium

Plant Number	Date	Number of microscopic fields counted	Total number of pollen grains	Number of grains germinated	% Germination	
					Plate 1	Plate 2
S_3.33.4 (4-5)	3/7/36	80	205	24	11.71	-
	17/7/36	200	1708	470	29.17	25.43
	31/7/36	150	410	67	19.28	7.69
	14/8/36	158	652	78	13.39	7.55
	28/8/36	no flowers available				
S_1.28.3 (9-11)	3/7/36	200	1863	937	47.83	52.38
	17/7/36	200	3584	1662	44.03	48.33
	31/7/36	200	2236	1233	55.70	54.54
	14/8/36	200	3338	1633	45.91	51.87
	28/8/36	200	2347	1112	49.82	45.09
S_1.32.32 (47-5)	3/7/36	200	3069	2611	83.93	86.40
	17/7/36	200	2379	1974	84.10	81.63
	31/7/36	200	1722	1433	81.19	84.51
	14/8/36	200	2591	2170	83.47	84.09
	28/8/36	no flowers available				

Table 11

Selected characteristics of persons 25 years old and over, by sex, race, and Spanish origin: March 1979

(Numbers in thousands. Persons of Spanish origin may be of any race)

Characteristic	Total	White	Black	Spanish origin
Total persons				
Median years of school completed	12.5	12.5	12.1	11.4
Percent high school graduate or more	66.5	68.1	51.2	44.1
Percent college graduate or more	16.2	17.0	9.4	8.1
Male				
Median years of school completed	12.5	12.5	12.0	11.4
Percent high school graduate or more	67.3	68.9	49.8	44.1
Percent college graduate or more	18.2	19.1	9.1	8.9
Female				
Median years of school completed	12.5	12.5	12.2	11.4
Percent high school graduate or more	65.8	67.4	52.4	44.1
Percent college graduate or more	14.4	15.1	9.6	7.4

TABLE II (Continued)

Plant Number	Date	Number of microscopic fields counted	Total number of pollen grains	Number of grains germinated	% Germination	
					Plate 1	Plate 2
I.31.9 (21-23)	3/7/36	200	2908	2444	82.94	85.51
	17/7/36	200	2103	1845	87.94	87.46
	31/7/36	200	1289	1166	92.41	88.66
	14/8/36	200	2149	1982	92.08	92.37
	28/8/36	200	1949	1712	87.74	87.93
S_2.32.29 (40-10)	3/7/36	200	2400	2081	87.70	85.85
	17/7/36	200	1724	1578	91.05	92.06
	31/7/36	200	1397	1252	86.05	90.95
	14/8/36	200	2325	2103	91.29	89.65
	28/8/36	200	1609	1303	81.71	80.33

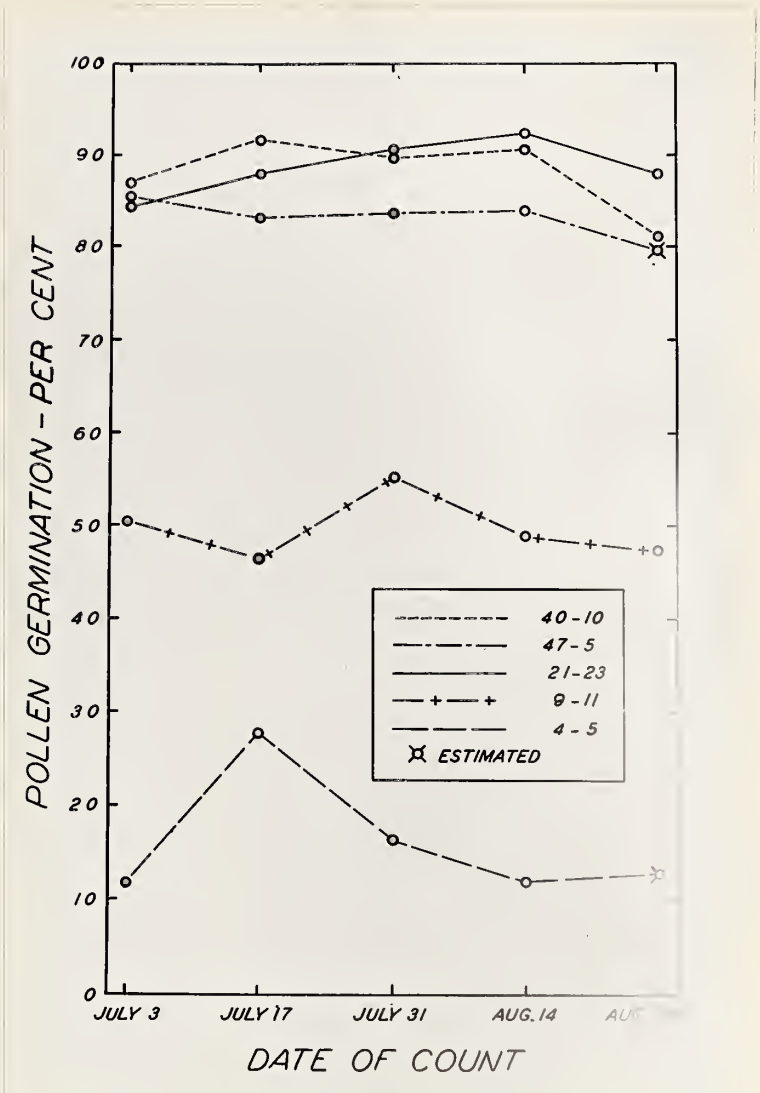

Figure 1

Pollen viability throughout season, expressed as percentage germination on agar-sugar medium

For purposes of analysis, the inverse-sine transformation was applied. Further, it was thought advisable to make two analyses because one count was incomplete and two others were missing. Table III gives the transformed data for all five plants at three different dates, and Table IV is for three plants at the five dates.

The analysis of variance, in degrees, for five plants at three dates is:

Variance due to	D.F.	M.S.	F	5% point
Plants	4	2,634.3877	92.05	3.84
Dates	2	5.5908	0.20	
Plants x Dates	8	28.6204		
Residual	15	6.6708		
Total	29			

The analysis of variance, in degrees, for three plants at five dates is:

Variance due to	D.F.	M.S.	F	5% point
Plants	2	2,129.2827	170.13	4.46
Dates	4	23.1048	1.85	3.84
Plants x Dates	8	12.5153		
Residual	15	2.5647		
Total	29			

It is clearly seen, from the two foregoing analysis tables, that differences in pollen viability due to dates is insignificant, while that for plants is highly significant. Therefore, it is concluded that,

TABLE III

Transformed percentage data from Table II, for five plants and three dates (expressed as degrees and obtained from transformation tables given by Bliss (4))

Plant Number	Date					
	17/7/36		31/7/36		14/8/36	
	Plate 1	Plate 2	Plate 1	Plate 2	Plate 1	Plate 2
S_3.33.4 (4-5)	32.71	30.26	26.06	16.11	21.47	16.00
S_1.28.3 (9-11)	41.55	44.03	48.27	47.58	42.65	46.09
S_1.32.32 (47-5)	66.50	64.60	64.30	66.81	66.03	66.50
I.31.9 (21-23)	69.64	69.30	74.00	70.36	73.68	74.00
S_2.32.29 (40-10)	72.64	73.68	68.11	72.54	72.84	71.28

TABLE IV

Transformed percentage data from Table II, for three plants and five dates (expressed as degrees, and obtained from transformation tables given by Bliss (4))

Plant Number		Date									
		3/7/36		17/7/36		31/7/36		14/8/36		28/8/36	
		Plate 1	Plate 2	Plate 1	Plate 2	Plate 1	Plate 2	Plate 1	Plate 2	Plate 1	Plate 2
S_1.28.3	(9-11)	43.74	46.38	41.55	44.03	48.27	47.58	42.65	46.09	44.89	42.19
I.31.9	(21-23)	65.57	67.62	69.64	69.30	74.00	70.36	73.68	74.00	69.47	69.64
S_2.32.29	(40-10)	69.47	67.94	72.64	73.68	68.11	72.54	72.84	71.28	64.67	63.65

under the conditions of this experiment, the pollen viability of a given plant does not vary significantly throughout the season.

Discussion.

The results obtained indicate that the seasonal variation in pollen viability for a given plant is not significant. This is in agreement with the work of Poole (26) for species of Crepis.

It is of interest to note that Poole (26) used plants grown in the greenhouse, whereas these studies were made on plants grown outside. The temperature and humidity would doubtless be more constant in the greenhouse than in the field.

In the field, temperature varied from 36° to 94°F., and the humidity from 20 percent to 100 percent during the course of the experiment. These varying conditions, however, had little effect on pollen viability, as shown by the results obtained.

Clarke and Fryer (15) grew clonal divisions of the same plant in the greenhouse under conditions of high and low temperatures. The actual temperatures used were not specified. They found that the temperature had no effect on the amount of poor pollen formed by the plant.

Results also indicate that the percentage of viable pollen produced by the individual plants may be, in part, responsible for the differences in pod-setting exhibited.

This same view has been expressed by several workers. Engelbert (20) was of the opinion that 22.5 percent sterile pollen was of little importance when a plant produced an abundance of pollen, but that the amount and sterility of pollen may be partly responsible for differences in seed-setting exhibited by different plants. Brink and Cooper (8) concluded that the amount of abnormal pollen may occasionally be large enough to limit seed-setting. Armstrong and White (3) believe that pollen sterility is a factor which influences the pod-setting and the number of seeds per pod. Bolton and Fryer (6) report that there is no general correlation between pollen viability and pod-setting, even though there seems to be a relationship in some instances.

B. Temperature Effect on Pollen Tube Growth

The relationship of temperature to pollen tube growth has been studied for several plant species, but as far as is known, there is no published report of studies made on alfalfa.

Literature Review.

Sandsten (27), using the pollen of apples and plums germinating on a cane sugar solution, obtained an increase in growth rate of the pollen tubes with an increase in temperature (86°-93.2°F.).

Working with Datura, Buchholz and Blakeslee (11) found that, within limits, the growth rate of pollen tubes increased as the temperature increased. They tested the tube growth in pistils, using a temperature range of 52° to 98.5°F. The growth rate increased steadily from 52° to 92°F., with a slight decline at 98.5°F.

Smith and Cochran (28) concluded that a temperature between 70° and 85°F. was optimum for pollen germination and tube growth in the tomato. Germination was poor at both 50° and 100°F.; and tube growth, while slow at both temperatures, was more limited and irregular at 100°F.

Cummings et al (18) found that on 4/10M sucrose- and glucose-agar media, the tubes of pear pollen grew more rapidly at 80° than at 58°F. They also found that, in the pistils, the growth rate of the tubes was less at 58° than at 80°F.

Methods.

Several tests were made using three different alfalfa plants. Due to the difficulty of staining pollen tubes in the styles, all samples were collected one-half hour after the flowers were tripped, and the tubes could be readily stained and measured while still in the stigma.

The temperatures used for this experiment were obtained in various places, and these will be referred to as "stations" in the text.

A temperature of 50°F. was obtained in a large cooling chamber equipped with a refrigeration unit. One of the 70°F. temperatures was obtained in the anteroom to the above mentioned cooling chamber. A series of three temperature cabinets in the greenhouse was maintained at 70°, 80° and 90°F. For the temperature of 100°F., an incubation oven in the laboratory was employed.

The temperatures fluctuated somewhat, but during the half-hour period when the tests were made, the temperature change was small. Temperatures recorded for the different "stations" were those registered at the time the tests were carried out.

Stem cuttings of the alfalfa plants in bottles of tap water were placed in the various "stations" at least twelve hours before the flowers were tripped. Ten to twelve flowers were tripped on each cutting and, after one-half hour, were removed from the temperature "stations" and treated in the following manner.

The pistil was dissected out from the surrounding floral parts and the stigma and style removed in one piece by clipping with a pair of scissors. This portion was then placed on a microscopic slide in a killing and staining solution, a cover-slip applied, and by pressure on the cover-slip the stigma was slightly crushed.

The killing and staining solution was suggested by Armstrong and White (3). It consisted of lacto-phenol, to which was added a small amount of an acid fuchsin - light green stain. The stain was made up of "8 parts of 1 percent aqueous acid fuchsin and 2 parts of 1 percent light green in 95 percent alcohol." (Armstrong and White (3)).

Six stigmas were collected at each temperature for a single determination. Measurements of the pollen tubes were made with the aid of an ocular micrometer, at a magnification resulting from the use of a 4 mm. objective in combination with 10x oculars. Measurements were recorded as units of the micrometer scale and later changed to microns.

Experimental Results.

The results obtained for the experiments are presented in Table V. These results are summarized in Table VI, and a graphic representation is to be found in Figure 2.

In no case did the pollen germinate at 50°F. in the allotted half-hour period. A slight bulging at the germ pores was seen in most instances, but no real pollen tubes had been formed. At all other temperatures, germination appeared to be quite normal, though no counts were made. It is to be regretted that no germination counts were taken, and also that it was not possible to test the tube growth at 60°F.

The results indicate that there is a linear relationship between the length of the pollen tubes and the temperature, this relationship holding for temperatures from 70° to 100°F. for the half-hour period.

An examination of the mean tube lengths with their standard errors (Table V), would lead to either one of two inferences. Firstly, that tube length is more variable as the mean length increases; or secondly, that higher temperatures cause more variability in tube length. Neither of these inferences can be confirmed by reference to data given in Table V, but tube measurements to be reported later (see Table VII) indicate that the former is the more justifiable.

TABLE V

Temperature effect on pollen tube growth during half-hour periods

Plant Number	Date	Temperature °F.	"Station"	Number of stigmas	Number of tubes measured	Mean length of tubes (microns)
I.31.9 (21-23)	28/7/39	72	chamber	6	40	31.92 ± 1.283*
	28/7/39	80	cabinet	6	29	40.46 ± 2.314
	28/7/39	90	cabinet	5	32	68.11 ± 3.065
	29/7/39	72	chamber	5	41	28.39 ± 1.182
	29/7/39	80	cabinet	5	37	43.72 ± 2.335
	29/7/39	90	cabinet	2	12	53.70 ± 6.166
S_1.32.32 (47-5)	1/8/39	50	chamber	6	0	no germination
	1/8/39	70.5	chamber	4	24	22.16 ± 1.122
	1/8/39	70	cabinet	4	33	23.39 ± 1.501
	1/8/39	80	cabinet	6	36	41.80 ± 2.321
	1/8/39	90	cabinet	6	54	58.22 ± 2.653
	3/8/39	50	chamber	6	0	no germination
	3/8/39	70	chamber	6	43	20.85 ± 0.706
	3/8/39	70	cabinet	4	33	22.66 ± 1.241
	3/8/39	84	cabinet	3	22	50.71 ± 3.071
	3/8/39	90	cabinet	2	5	58.37 ± 4.650
	4/8/39	100	oven	5	38	91.84 ± 4.812
	5/8/39	100	oven	3	22	91.06 ± 6.659

Tableau 4

Nombre des visites de bateaux aux ports-pays étrangers

TABLE V (Continued)

Plant Number	Date	Temperature °F.	"Station"	Number of stigmas	Number of tubes measured	Mean length of tubes (microns)
I.28.18 (14-38)	4/8/39	50	chamber	5	0	no germination
	4/8/39	71.6	chamber	5	28	22.15 ± 1.148
	4/8/39	70	cabinet	4	23	21.81 ± 1.450
	4/8/39	82.4	cabinet	6	104	35.90 ± 1.305
	4/8/39	90	cabinet	5	48	52.56 ± 2.873
	4/8/39	100	oven	4	29	68.87 ± 4.845
	5/8/39	50	chamber	4	0	no germination
	5/8/39	70	cabinet	4	31	21.08 ± 0.857
	5/8/39	100	oven	2	15	58.78 ± 3.710

* Standard Error of the mean.

TABLE VI

Summary of data on temperature effect on pollen tube growth

Plant Number	Temperature °F.	Number of tubes measured	Mean (weighted) length of tubes (microns)
I.31.9 (21-23)	72	81	30.14
	80	66	42.28
	90	44	64.19
S_1.32.32 (47-5)	70	109	22.17
	70.5	24	22.17
	80	36	41.80
	84	22	50.71
	90	59	58.22
	100	60	91.55
I.28.18 (14-38)	70	53	21.80
	71.6	28	22.15
	82.4	104	35.89
	90	48	52.57
	100	44	65.43

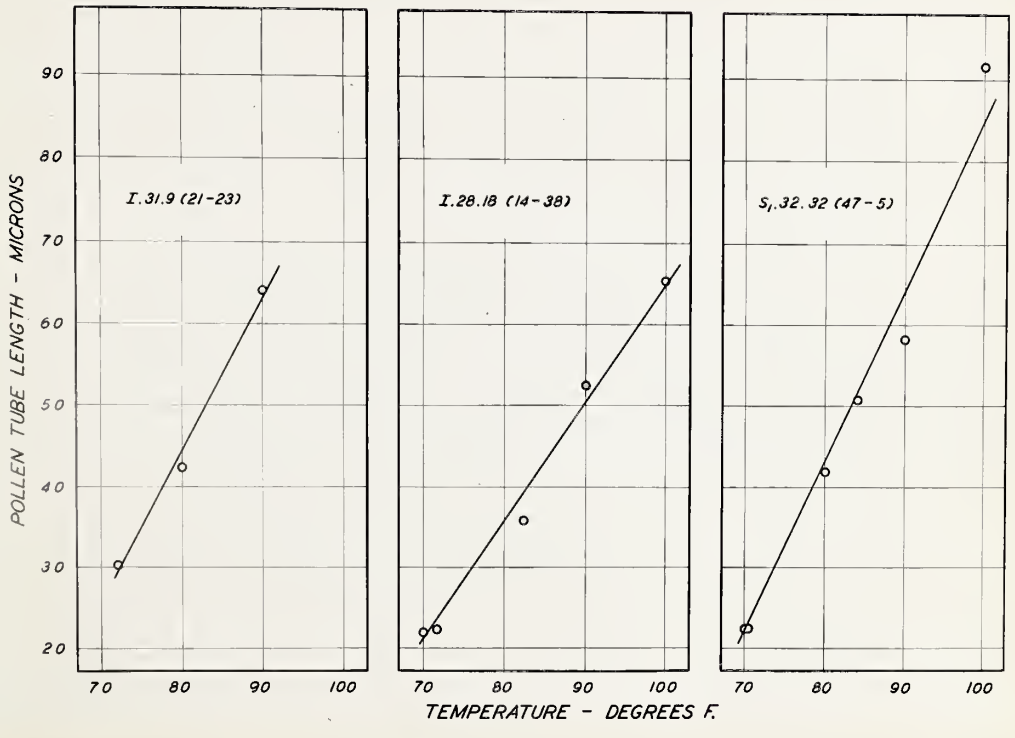

Figure 2

Temperature effect on pollen tube growth
during half-hour periods

Microphotographs were made from the prepared material for purposes of illustration, and these are to be seen in Figures 3 to 14.

Discussion.

The results obtained in the aforementioned studies indicate that pollen tubes grow more rapidly as temperature increases. It must be remembered, however, that in the half-hour test period, two distinct processes occurred. These were germination and pollen tube elongation. As has been shown by other workers for different plant species, the germination rate is affected by temperature, being more rapid at higher temperatures. Therefore, a true picture of tube growth is not presented, as tube elongation was doubtless in progress for different lengths of time at the various temperatures.

It is of interest to note the tube growth at 100°F. (Table VI, Figures 8 and 14), which appeared to be quite normal. At this same temperature, Smith and Cochran (28) found the tube growth of tomato to be very poor; the length, even after 54 hours, not being as great as the length attained in 12 hours at temperatures of 70° and 85°F.

Figure 3

Pollen tube growth at 50°F. (chamber) during a half-hour period. x129

No germination

Plant I.28.18 (14-38)

Figure 4

Pollen tube growth at 70°F. (cabinet) during a half-hour period. x129

Grain with tube corresponding to mean length marked by a white dot

Plant I.28.18 (14-38)

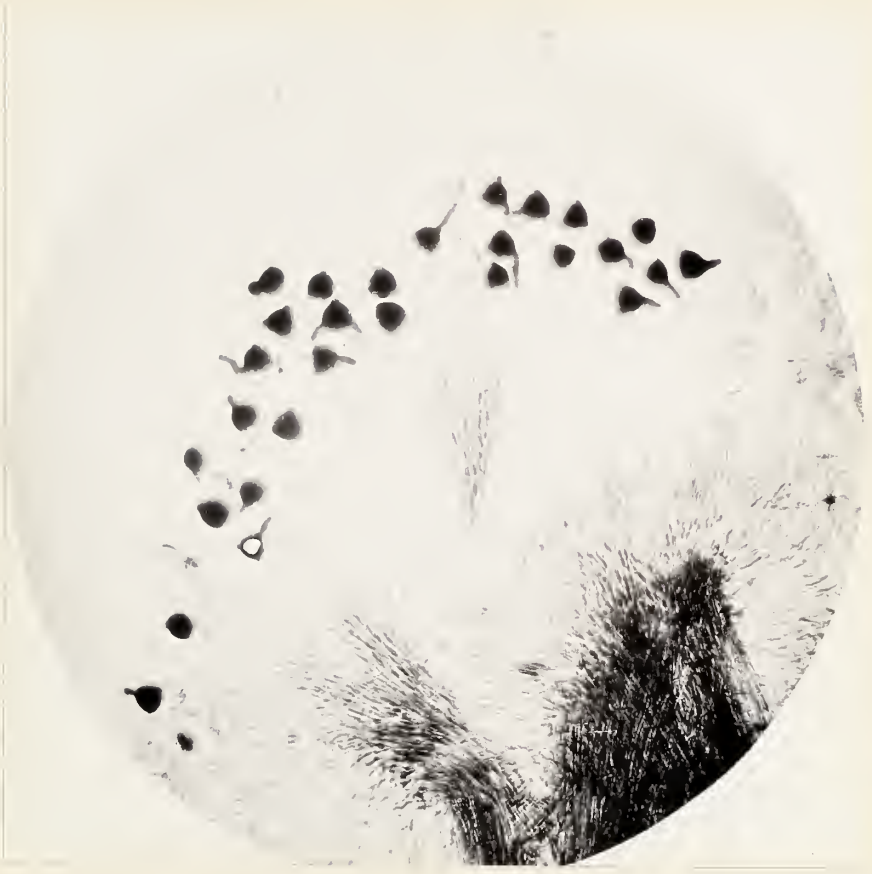

Figure 5

Pollen tube growth at 71.6°F. (chamber) during a half-hour period. x129

Grain with tube corresponding to mean length marked by a white dot

Plant I.28.18 (14-38)

Figure 6

Pollen tube growth at 82.4°F. (cabinet) during a half-hour period. x129

Grain with tube corresponding to mean length marked by a white dot

Plant I.28.18 (14-38)

Figure 7

Pollen tube growth at 90°F. (cabinet) during a half-hour period. x129

Grain with tube corresponding to mean length marked by a white dot

Plant I.28.18 (14-38)

Figure 8

Pollen tube growth at 100°F. (oven) during a half-hour period. x129

Grain with tube corresponding to mean length marked by a white dot

Plant I.28.18 (14-38)

Figure 9

Pollen tube growth at 50°F. (chamber) during a half-hour period. x129

No germination

Plant S_1.32.32 (47-5)

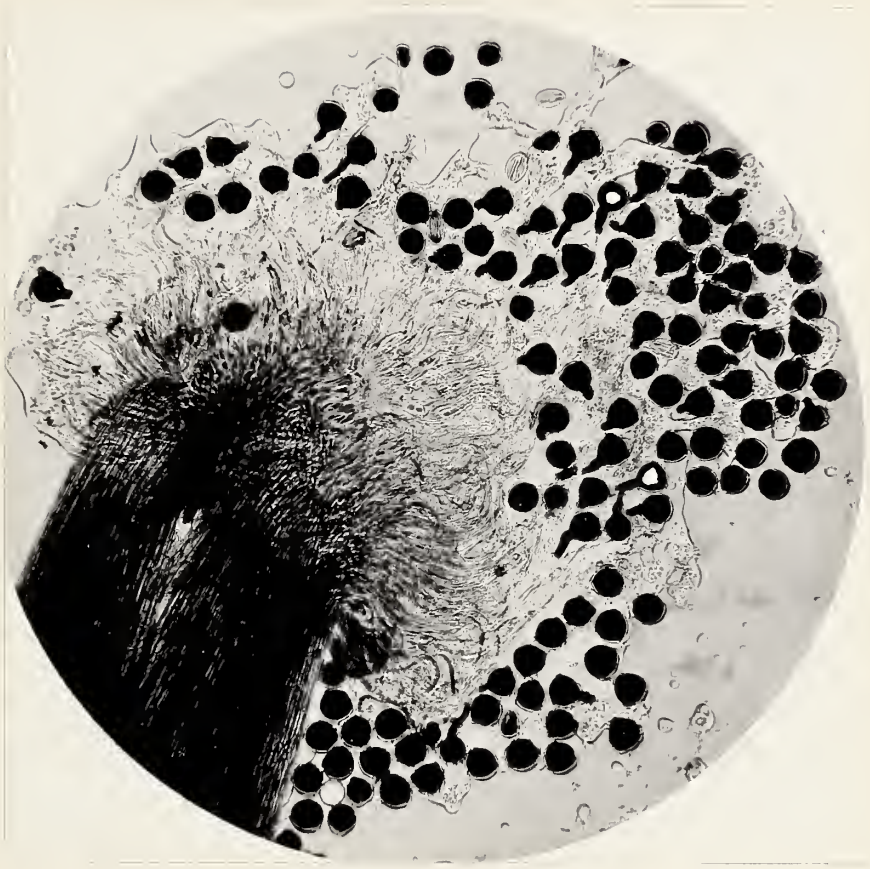

Figure 10

Pollen tube growth at 70°F. (chamber) during a half-hour period. x129

Grain with tube corresponding to mean length marked by a white dot

Plant S_1.32.32 (47-5)

Figure 11

Pollen tube growth at 70°F. (cabinet) during a half-hour period. x129

Grain with tube corresponding to mean length marked by a white dot

Plant S_1.32.32 (47-5)

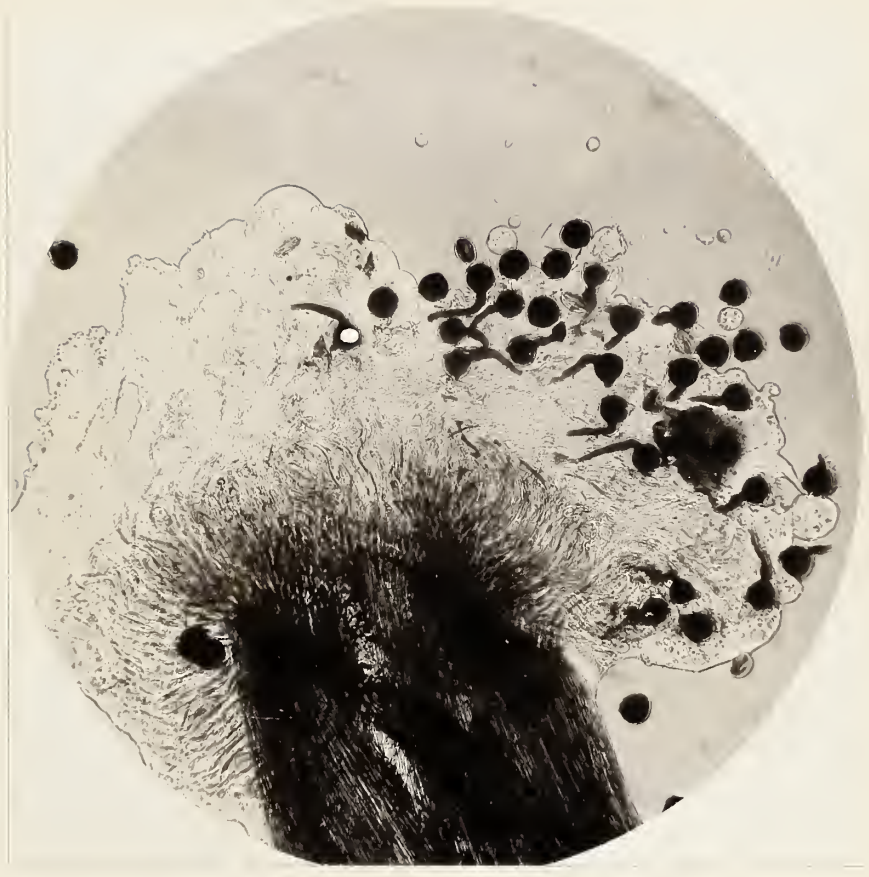

Figure 12

Pollen tube growth at 84°F. (cabinet) during a half-hour period. x129

Grain with tube corresponding to mean length marked by a white dot

Plant S_1.32.32 (47-5)

Figure 13

Pollen tube growth at 90°F. (cabinet) during a half-hour period. x129

Grain with tube corresponding to mean length marked by a white dot

Plant S_1.32.32 (47-5)

Figure 14

Pollen tube growth at 100°F. (oven) during a half-hour period. x129

Grain with tube corresponding to mean length marked by a white dot

Plant S_1.32.32 (47-5)

The three plants tested in this experiment were classed as fertile, and each set pods quite freely on selfing. The reaction of the pollen tubes of poorer pod-setting plants to temperature is not known. It may be that the tubes of such plants behave in a somewhat different manner.

C. Pollen Germination and Tube Growth in Different Atmospheres

In 1869, Van Tieghem (Brink (7)) demonstrated the necessity of oxygen for pollen germination. The reaction of pollen to different concentrations of oxygen in the atmosphere had, however, not been tested, and so experiments were conducted, first proving the necessity of oxygen for pollen germination in preliminary tests.

If, as is believed by Carlson (12) and several others, seed-pods are formed from untripped alfalfa flowers, then the pollen must have germinated. However, no direct evidence is found in the literature to prove that pollen germination does occur in untripped flowers.

Some investigators are of the opinion that tripping is an absolute pre-requisite to pod formation (for example, Armstrong and White (3)), under certain climatic conditions. At Edmonton, tripping appears to

be necessary for pollen germination in the alfalfa flower. If this is the case, why does the pollen not germinate in the untripped flower? The work of Martin (23) would indicate that moisture supply is a prime factor in pollen germination.

It was thought that the atmosphere inside the keel of the untripped flower might consist of a high concentration of carbon dioxide, which could possibly inhibit pollen germination. Therefore, tests were made to determine the effect of carbon dioxide on pollen germination.

Methods.

To prove the necessity of oxygen for pollen germination and tube growth, two different methods were employed. In the first, a Spray jar was used, a raceme of flowers being suspended in the jar and the jar sealed before mixing the pyrogallic acid and dilute potassium hydroxide to remove the oxygen. A decreased pressure resulted inside the jar, and lest this be detrimental, another method was tried. For the second method a 250 cc. suction flask was used in such a way that the oxygen, as it was exhausted by the pyrogallic acid - potassium hydroxide mixture, was replaced by oxygen-free air.

For tests made in 1938 regarding the effect of carbon dioxide on pollen germination and tube growth, the gas was generated from marble chips and dilute hydrochloric acid. A suction flask was thoroughly flushed with carbon dioxide before a raceme of tripped flowers was enclosed, and the gas flow was continued for several minutes after the plant material was placed in the flask. In these tests a constant humidity of 45 percent was maintained in designated flasks by placing a saturated solution of potassium nitrite in flasks and immersing in a water bath at 20°C. Tests were made when the atmospheric humidity was approximately 45 percent so that a check could be used with no potassium nitrite solution in the flask.

The atmospheres containing different percentages of oxygen and carbon dioxide were prepared in five gallon (wine measure) bottles. Oxygen and nitrogen were run from gas cylinders into the bottles by displacement of water. Carbon dioxide, from a cylinder, and air were put into the bottles in the same manner. Bottles were placed near a temperature oven over night to facilitate mixing of the gases.

The gas mixtures were forced through the suction flasks, in which the plant material was placed, by displacement with water. In the case of the carbon dioxide mixtures, CO_2-saturated water was used for displacement.

The test materials for the oxygen tests were racemes of tripped flowers; that for the carbon dioxide tests, pollen spread on an agar-sugar medium. When flowers were used, the pistils were prepared and examined by the methods outlined in section B. In the case of the carbon dioxide tests, only germination counts were taken. The agar-sugar medium was poured in a thin film on microscopic slides, and when the pollen was ready for examination dilute methylene blue chloride was placed on the medium and a cover-slip applied.

Experimental Results.

Data obtained from tests made in Spray jars to prove the necessity of oxygen for pollen germination are presented in Table VII. No germination counts were taken, but pollen tube lengths were used for comparisons.

In all cases, tube growth was significantly less when oxygen was removed from the atmosphere. The 6-hour test period used on August 18, 1938, was found to be too long. The tubes for the checks had grown to such a length as to make accurate measurements impossible.

TABLE VII

Effect of removing oxygen from the atmosphere in which pollen was germinating

Plant Number	Date	Treatment	Time of treatment (hours)	Number of tubes measured	Mean tube length (microns)
S_2.32.26 (34-5)	18/8/38	Pyrogallic acid + potassium hydroxide	6	35	30.43 ± 3.240*
	18/8/38	Potassium hydroxide	6	17	163.52 ± 19.706
	18/8/38	Check	6	16	218.76 ± 31.568
	22/8/38	Pyrogallic acid + potassium hydroxide	2	49	5.86 ± 0.310
	22/8/38	Potassium hydroxide	2	50	130.48 ± 7.473
	22/8/38	Check	2	50	145.28 ± 11.706
I.31.9 (21-23)	22/8/38	Pyrogallic acid + potassium hydroxide	2	25	5.23 ± 0.373
	22/8/38	Check	2	39	149.70 ± 11.212
S_3.33.9 (6-33)	22/8/38	Pyrogallic acid + potassium hydroxide	2	9	3.37 ± 0.224
	22/8/38	Potassium hydroxide	2	43	140.90 ± 8.229
	22/8/38	Check	2	50	137.96 ± 9.835

* Standard Error of the mean.

The standard error of the mean tube length is greatest for the longer tubes. The data presented, though inadequate, may help to answer the question which was raised when discussing the effect of temperature on tube growth under section B (page 18).

The second method for removing oxygen from the atmosphere was tested, using plant S_2.32.26 (34-5). For comparison, a test was also made in a Spray jar. During a 5-hour test period, there was absolutely no germination in the suction flask freed of oxygen, and so no tubes could be measured. In the Spray jar there was some germination, and the mean length of 16 pollen tubes was 4.47 $\pm$ 0.291 microns.

The necessity of oxygen for pollen germination was proven in these tests. It may also be concluded that the second method used removed oxygen from the air more efficiently.

A summary of results obtained from tests to determine the effect of different concentrations of oxygen on pollen germination and tube growth is given in Table VIII.

The test period in this experiment was one-half hour, and for each stigma used a germination percentage was recorded. At the suggestion of Dr. C. H. Goulden*,

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* Correspondence with Dr. Goulden, Senior Agricultural Scientist, Dominion Rust Research Laboratory, Winnipeg, Manitoba.

TABLE VIII

Pollen germination and tube growth as affected by variations in oxygen content of the atmosphere (using plant S_1.32.32 (47-5))

Percentage oxygen	Date	Number of stigmas	Total number of pollen grains	Number of grains germinated	Percentage germination	Number of pollen tubes measured	Mean length of tubes (microns)
0 (100% nitrogen)	15/7/39	6	92	0	0	--	-
0 (100% nitrogen)	21/7/39	6	84	0	0	--	-
5	15/7/39	6	100	77	77.00	65	57.15 ± 3.029*
10	15/7/39	6	103	86	83.50	54	52.08 ± 3.159
20 (air)	15/7/39	5	200	140	70.00+	87	52.38 ± 2.008
20 (air)	21/7/39	5	160	135	84.38	61	48.64 ± 1.981
20 (air)	25/7/39	6	109	83	76.15	52	51.39 ± 3.620
30	21/7/39	6	137	120	87.59	47	49.48 ± 2.533
40	21/7/39	6	202	156	77.23	71	41.71 ± 1.985
50	25/7/39	6	167	84	50.30	62	45.36 ± 2.457
60	25/7/39	6	137	65	47.45	46	58.16 ± 3.121
70	25/7/39	6	174	98	56.32	74	45.48 ± 2.373

* Standard Error of the mean.

\+ Humidity in flask was low which probably helps account for lower germination.

an analysis of variance was made of the percentage data for the different stigmas, recorded in Table IX. The result of the analysis is as follows:

Variance due to	D.F.*	Variance	F	5% point
Treatment	7	1,168.28	6.93	2.26
Residual	40	168.51		
Total	47			

* No corrections made for the missing value.

A difference between means of 15.15 percent is necessary for significance. It may therefore be concluded that, in this experiment, over 40 percent oxygen in the atmosphere has a detrimental effect on pollen germination and, further, that some oxygen is absolutely necessary for germination.

The mean lengths of pollen tubes at the different oxygen concentrations are quite variable. This variability appears to bear no relation to oxygen concentration, and the most probable explanation is a reaction to temperature. If this is true, the following generalization could be made. Differences in oxygen content of the atmosphere, between 5 and 70 percent, have no effect on the length that the pollen tubes attain.

The results obtained in the preliminary experiments with carbon dioxide are given in Table X.

TABLE IX

Percentage pollen germination on stigmas in atmospheres of different oxygen concentrations

Stigma Number	Percentage oxygen							
	5%	10%	20%	30%	40%	50%	60%	70%
1	70.59	96.00	82.86	100.00	80.56	73.91	80.00	84.62
2	78.57	66.67	85.29	85.71	80.95	44.44	72.73	57.69
3	100.00	83.87	92.86	88.89	86.67	37.50	62.50	42.86
4	92.31	100.00	78.57	94.74	89.47	47.27	45.16	58.18
5	75.00	76.00	85.72	85.72	64.06	72.73	27.27	50.00
6	71.43	66.67	80.04*	82.86	81.25	58.33	44.23	70.59

* Estimated mixing value.

| | | |

TABLE X

Pollen germination and tube growth as affected by carbon dioxide
(using plant S_2.32.26 (34-5))

Treatment	Date	Duration of test (hours)	Number of pollen tubes measured	Mean length of tubes (microns)	Remarks
Carbon dioxide over potassium nitrite solution	26/8/38	1	0	-	few grains showed any evidence of germination.
Air over potassium nitrite solution	26/8/38	1	70	96.45 ± 4.067*	good germination.
Air	26/8/38	1	53	92.58 ± 4.917	good germination.
Carbon dioxide over potassium nitrite solution	27/8/38	¾	6	3.04 ± 0.000	poor germination.
Air over potassium nitrite solution	27/8/38	¾	56	39.74 ± 2.205	good germination.
Air	27/8/38	¾	84	47.95 ± 2.623	good germination.
Carbon dioxide over potassium nitrite solution	29/8/38	¾	5	3.04 ± 0.000	poor germination.
Air over potassium nitrite solution	29/8/38	¾	37	57.43 ± 1.342	good germination.

* Standard Error of the mean.

These results indicate that carbon dioxide has a very detrimental effect on pollen germination, and that it also retards pollen tube growth.

The difference in pollen tube length found in the checks on August 27, 1938, is statistically significant. This difference was due to temperature, and not to the presence of potassium nitrite solution in the flask.

Table XI contains results obtained from tests made to determine the effect of varying the carbon dioxide concentrations of the atmospheres in which pollen was germinating. As the concentration increased, pollen germination decreased, and no germination occurred in 40 percent carbon dioxide. It was noted that the pollen tubes became shorter as the carbon dioxide increased, till at 30 percent the tubes were very short and abnormal in shape.

Discussion.

The necessity of oxygen for pollen germination has been proven. The occurrence of germination when oxygen was exhausted from a closed vessel was probably due to a comparatively slow uptake of oxygen by the pyrogallic acid - potassium hydroxide mixture.

TABLE XI

Pollen germination on an agar-sugar medium, as affected by changes in the concentration of carbon dioxide in the atmosphere (using plant S_1.32.32 (47-5))

Percentage carbon dioxide	Date	Number of microscopic fields counted	Number of pollen grains counted	Number of pollen grains germinated	Percentage germination
0 (air)	9/8/39	22	348	268	77.01
0 (air)	11/8/39	21	580	424	73.10
5	9/8/39	50	1451	1007	69.40
10	9/8/39	38	1276	631	49.45
15	9/8/39	33	1143	438	38.32
20	11/8/39	38	1226	153	12.48
30	11/8/39	39	1071	21	1.96
40	11/8/39	30	1225	0	0.00

Germinating alfalfa pollen seems to be able to withstand a wide range of oxygen concentration without any detrimental effects. It is quite possible that the method of testing was not the best, and the germination percentages obtained may be far from accurate. This same test might well be repeated, using an agar-sugar medium for germination.

Pollen germination is greatly reduced in an atmosphere containing 10 percent carbon dioxide. The actual significance of this fact is not known, but the concentration of carbon dioxide in the keel of the alfalfa flower may be great enough to stop germination.

Moisture conditions on the artificial medium used for the reported tests, were far better than would be found in the sheathing petals of an untripped alfalfa flower. Pollen germination in the untripped flowers may, then, be prevented by a combination of low moisture conditions and a high concentration of carbon dioxide.

PART II. TEMPERATURE EFFECT ON POD- AND SEED-SETTING

Introduction

The effect of temperature on pod-setting is referred to, time after time, in the literature. However, no critical studies have been carried out under controlled conditions.

During the past seventeen years, selection of alfalfa for an increase in seed-setting has been progressing at the University of Alberta. Selection of superior plants has been based on a system of scoring, scores ranging from 0 to 5. This method of scoring lacks precision and is of limited value, hence means of obtaining a reliable fertility index of different plants have been sought.

Several methods were tested in 1936, and the use of stem cuttings in tap water showed the most promise. The proper conditions for conducting such tests were then considered and, in 1937, stem cuttings were tested at different temperatures. The results obtained, though of no great value for the establishment of a fertility index, indicated that temperature influences pod- and seed-setting. Further tests were conducted in 1939, and are to be reported together with the earlier tests.

To establish the relationship between pod-setting on cuttings and on the plant itself, preliminary tests were carried out in 1939. The results are somewhat contradictory, but will be reported with full realization that much more work needs to be done before any definite conclusions can be reached.

Literature Review

Aicher (1) believed that warm, dry, sunshiny weather was the best for pod-setting. Alter (2), from an extensive study of weather conditions in the seed producing areas of Utah, concluded that the proper combination of spring and summer conditions was of prime consideration; warm springs and cool summers resulting in the best seed-setting. Mean monthly maximum temperatures above 90°F. during the blossoming period were unfavorable, and short periods with temperatures above 100°F. when moisture was lacking resulted in light yields of seed. He also concluded that winds, combined with excessively high temperatures at times when moisture was deficient, caused stripping of the flowers. The detrimental effect of hot, dry winds has also been mentioned by numerous other authors

(13, 22, 24, 30). Cool, damp weather is considered to be unfavorable for fertilization and seed formation (22, 24, 32, 33). Carlson (13), working in Utah, found that the highest pod-setting occurred when atmospheric humidity ranged from 36 to 65 percent, at temperatures between 72° and 89°F., and the greatest amount of stripping took place in sultry weather, or when the relative humidity was greater than 70 percent and the temperature above 90°F.

Various factors affect the number of seeds which are produced in each alfalfa pod. Results obtained by several workers prove that more seeds are formed per pod from cross-pollinations than from self-pollinations (9, 19, 25, 31). Plants differ in their ability to set a high number of seeds per pod, and this fact has been demonstrated on numerous occasions (3, 5, 6, 17). Bolton and Fryer (6) found that, when the pod-setting of eleven test plants decreased by an average of 22.3 percent from one date to another, the decrease in number of seeds per pod was 7.6 percent. Thus they concluded that external conditions may affect the number of seeds produced per pod.

Embryo abortion results in a difference in the number of normal seeds per pod at maturity. Woodworth (34) found that different varieties of soybeans produced from 9.4 to 22.2 percent aborted seeds.

Cooper, Brink and Albrecht (17) found that in five high seed-setting alfalfa plants an average of 3.1 ovules per flower were fertilized, while only 1.25 seeds per flower were found at maturity; and in five low seed-setting plants 2.5 ovules were fertilized and only 0.07 seeds per flower developed to maturity.

Brink and Cooper (9) report that 144 hours after pollination, 34 percent of the self-fertilized ovules had collapsed, while 7 percent of the ovules developing from cross-fertilization had collapsed. Bolton (5) found that there were plant differences in the amount of embryo abortion. Martin (23) thought that the failure of fertilized ovules to develop to maturity was the result of drought conditions.

Brink and Cooper (10) suggest the term "somatoplastic sterility" for the condition commonly referred to as embryo abortion. From an extensive histological study of developing fertile ovules, these workers concluded that the condition was the result of unequal growth rates of the different parts of the ovules. They believed that if the growth rate of the maternal tissue was more rapid than that of the endosperm, the endosperm would not be able to obtain sufficient food material and so would starve, and the ovule therefore collapse.

Methods

Stem cuttings were taken from the plants, cut under water, and placed in pint bottles of tap water. The old flowers and unopened buds were carefully removed from each raceme, and the remaining flowers tripped with the aid of forceps. Each raceme was tagged, and a record made of the number of flowers tripped. The same procedure was followed in all cases in which stem cuttings were used. When flowers were tripped on the plants themselves, the racemes were treated in a similar manner.

For testing the effect of temperature on pod-setting, bottles containing cuttings, on which the flowers had been tripped, were placed in temperature cabinets (page 16). Two plants were tested at a time, and for each temperature or temperature change used, two bottles were prepared. The first set of tests conducted in 1937 involved temperatures of 100°, 85° and 70°F., all others in 1937 and 1939 were carried out at 90°, 80° and 70°F. In some cases, the cuttings were maintained at a given temperature throughout the test period of 12 to 14 days. In other cases, material was transferred from one temperature to another, 24 hours after the flowers were tripped. For example, 90-80°F.

implies that cuttings were at 90°F. for 24 hours immediately after tripping, and at 80°F. for the remainder of the period.

A large pan of water was placed in each cabinet to reduce the variation in atmospheric humidity. The tap water in the bottles was changed every two days throughout the test period.

Pods were counted and recorded for each raceme, and when the pods were saved for seed counts, they were placed in small vials of 70 percent alcohol. The alcohol decolorized the pods, and by viewing pods in transmitted light the seeds could readily be counted. The numbers of normal and aborted seeds were recorded for each pod.

The method used to compare pod-setting on plants and on cuttings is illustrated in Figure 15. The stems of plants were held upright by wire supports, and the stem cuttings in bottles were placed at the same height by attaching the bottles to wooden stakes.

The normal seeds were dissected from the pods, dried in an oven at 80°C. for 14 hours, and weighed. Before weighing, photographs were taken to illustrate the size attained by seeds at the various temperatures.

Figure 15

Pod-setting compared on plant (left) and stem cuttings (right), illustrating the method for placing tripped flowers at the same level

Experimental Results

Results obtained for pod-setting in the temperature cabinets during 1937 are given in Table XII, and a graphic representation of these results is to be found in Figure 16. In each graph, the solid line is the calculated best-fitting line for the pod-setting percentages at temperatures of 70°, 80° and 90°F.

The pod-setting data indicate that, as temperature decreases, pod-setting increases. Plant S_1.32.32 (47-5) did not respond to changes in temperature, the pod-set being very good at all temperatures.

The analysis of variance of data for the test of June 24, 1937, expressed in degrees, is as follows:

Variance due to	D.F.	Variance	F	5% point
Temperatures	3	117.2697	10.22	9.28
Plants	1	280.9352	24.48	10.13
Residual	3	11.4755		
Total	7			

Both temperatures and plant differences are significant. The summary for temperature means is given in Table XIII.

TABLE XII

Pod-setting results from tests conducted on stem cuttings in controlled temperature cabinets during 1937

Plant Number	Date	Temperature °F.	Number of flowers tripped	Number of pods set	Pod-setting (%)	Inverse-sine transformation (degrees)
S_2.32.26 (33-4)	24/6/37	100	127	0	0	-
		100-85	103	47	45.63	42.48
		85	104	64	61.54	51.65
		85-70	151	101	66.89	54.88
		70	128	89	69.53	56.48
I.31.9 (21-35)	24/6/37	100	117	0	0	-
		100-85	102	24	23.53	29.00
		85	111	35	31.53	34.14
		85-70	112	63	56.25	48.62
		70	132	69	52.27	46.32
S_2.32.26 (33-4)	7/8/37	90	107	13	12.15	20.36
		90-80	120	32	26.67	31.11
		80	109	48	44.04	41.55
		90-70	107	60	56.07	48.50
		80-70	105	60	57.14	49.08
		70	115	82	71.30	57.61

TABLE XII (Continued)

Plant Number	Date	Temperature °F.	Number of flowers tripped	Number of pods set	Pod-setting (%)	Inverse-sine transformation (degrees)
I.31.9 (21-35)	7/8/37	90	114	11	9.65	18.15
		90-80	113	17	15.04	22.79
		80	102	41	40.20	39.35
		90-70	106	22	20.75	27.13
		80-70	123	47	38.21	38.17
		70	104	46	44.23	41.67
S_2.32.7 (10-34)	23/8/37	90	104	11	10.58	19.00
		90-80	103	26	25.24	30.13
		80	48	25	52.08	46.20
		90-70	106	32	30.19	33.34
		80-70	104	61	58.65	50.01
		70	79	29	36.71	37.29
S_1.32.32 (47-5)	23/8/37	90	80	43	53.75	47.18
		90-80	104	61	58.65	50.01
		80	108	63	58.33	49.78
		90-70	100	54	54.00	47.29
		80-70	110	56	50.91	45.52
		70	100	49	49.00	44.43

(Continued)

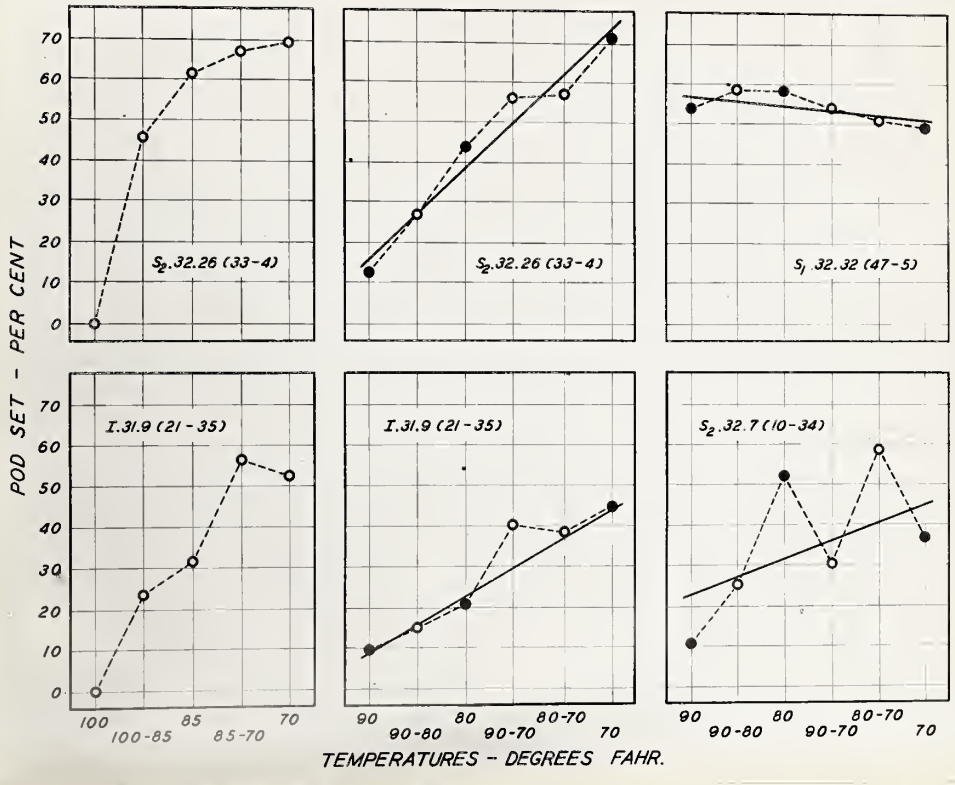

Figure 16

Temperature effect on pod-setting, 1937. Solid lines are the calculated best-fitting lines for temperatures of 70°, 80° and 90°F. indicated by solid circles.

TABLE XIII

Summary of pod-setting means, in degrees, for the different temperatures, and the corresponding percentage values (June 24, 1937)

	Temperatures (°F.)				S.E.	Difference for significance
	100-85	85	85-70	70		
Degrees	35.74	42.90	51.75	51.40	±2.395	10.777
Percentage	34.1	48.1	61.7	61.1		

The analysis of variance of data expressed in degrees, for the other plants tested in the temperature cabinets during 1937, omitting plant S_1.32.32 (47-5), is as follows:

Variance due to	D.F.	Variance	F	5% point
Temperatures	5	343.9981	10.90	3.33
Plants	2	154.9607	4.91	4.10
Residual	10	31.5484		
Total	17			

The data on temperature effects are summarized in Table XIV.

TABLE XIV

Summary of pod-setting means, in degrees, for the different temperatures, and the corresponding percentage values (1937)

	Temperatures (°F.)						S.E.	Difference for significance
	90	90-80	80	90-70	80-70	70		
Degrees	19.17	28.01	42.37	36.32	45.75	45.52	±3.092	9.624
Percentage	10.8	22.1	45.4	35.1	51.3	50.9		

The mean percentage pod-setting at 80°F. is much higher than might be expected, in view of the pod-setting obtained for plant S_2.32.7 (10-34) at this temperature. A possible explanation of this result is that the optimum temperature for pod-setting is not the same for all plants, and for plant S_2.32.7 (10-34) the optimum is close to 80°F.

In 1937, pods were saved only from tests made on June 24. The summary of the seed counts for these pods is presented in Table XV. The total number of seeds per pod does not appear to vary greatly at the different temperatures, but the number of normal seeds per pod is greater at 70°F. than at 85°F. This would indicate that embryo abortion occurs more frequently at the higher temperature.

Pod-setting data from the 1939 tests are given in Table XVI, and graphs for these data appear in Figure 17.

The analysis of variance of data expressed in degrees for the 1939 pod-setting data is:

Variance due to	D.F.*	Variance	F	5% point
Temperatures	5	598.9930	22.89	2.60
Plants	5	382.8896	14.63	2.60
Residual	25	26.1665		
Total	35			

* No corrections made for missing values.

TABLE XV

Temperature effect on seed-setting, 1937

Plant Number	Temperature °F.	Pod-setting (%)	Number of pods	Number of seeds			Seeds per pod		Seeds per flower	
				Normal	Aborted	Total	Total	Normal	Total	Normal
S_2.32.26 (33-4)	100-85	45.63	46	86	14*	100	2.17	1.87	0.99	0.85
	85	61.54	33	29	43	72	2.18	0.88	1.34	0.54
	85-70	66.89	101	232	15	247	2.45	2.30	1.64	1.54
	70	69.53	89	223	9	232	2.61	2.51	1.81	1.74
I.31.9 (21-35)	100-85	23.53	24	21	32	53	2.21	0.88	0.52	0.21
	85	31.53	31	28	42	70	2.26	0.90	0.71	0.28
	85-70	56.25	63	108	22	130	2.06	1.71	1.16	0.96
	70	52.27	69	162	0	162	2.35	2.35	1.23	1.23

* This set was counted first, and it is quite probable that the aborted ovules were not as accurately counted as for other sets.

TABLE XVI

Pod-setting results from tests conducted on stem cuttings in controlled temperature cabinets during 1939

Plant Number	Date	Temperature °F.	Number of flowers tripped	Number of pods set	Pod-setting (%)	Inverse-sine transformation (degrees)
I.28.18 (14-38)	4/7/39	90	168	4	2.38	8.91
		90-80	217	22	10.14	18.53
		80	231	72	31.17	33.96
		90-70	225	70	31.11	33.89
		80-70	221	91	41.18	39.93
		70	239	143	59.83	50.65
S_1.31.1 (23-4)	4/7/39	90	208	25	12.02	20.27
		90-80	214	22	10.28	18.72
		80	245	86	35.10	36.33
		90-70	209	52	24.88	29.93
		80-70	197	92	46.70	43.11
		70	181	75	41.44	40.05
I.31.9 (21-35)	19/7/39	90	239	29	12.13	20.36
		90-80	271	84	31.00	33.83
		80	206	35	16.99	24.35
		90-70	203	73	35.96	36.87
		80-70	241	89	36.93	37.41
		70	262	95	36.26	37.05

TABLE XVI (Continued)

Plant Number	Date	Temperature °F.	Number of flowers tripped	Number of pods set	Pod-setting (%)	Inverse-sine transformation (degrees)
S_2.32.7 (10-34)	19/7/39	90	104	4	3.85	11.39
		90-80	118	4	3.39	10.63
		80	118	21	17.80	24.95
		90-70	107	23	21.50	27.63
		80-70	113	36	31.86	34.39
		70	131	--	35.40*	36.51
I.31.9 (21-23)	31/7/39	90	177	70	39.55	39.00
		90-80	176	78	44.32	41.73
		80	110	59	53.64	47.06
		90-70	147	90	61.22	51.47
		80-70	158	111	70.25	56.98
		70	150	96	64.00	53.13
S_2.32.26 (34-5)	31/7/39	90	54	3	5.56	13.69
		90-80	99	-	22.53*	28.32
		80	76	21	27.63	31.69
		90-70	130	58	44.62	41.90
		80-70	130	67	51.54	45.86
		70	132	75	56.82	48.91

* Estimated missing values.

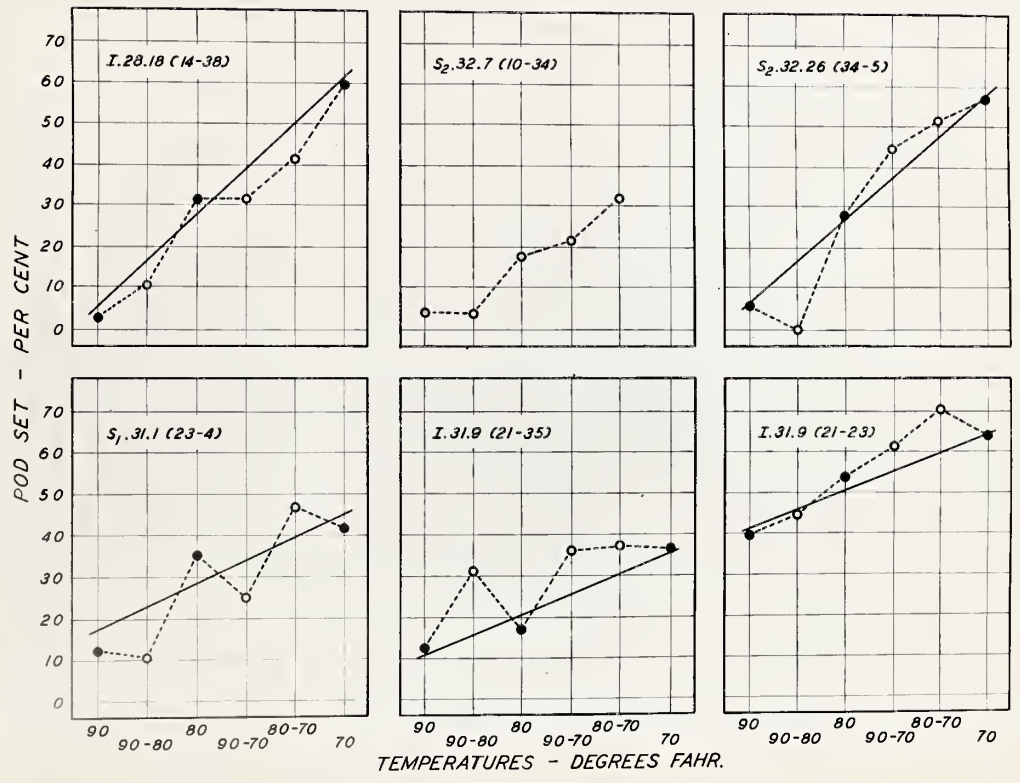

Figure 17

Temperature effect on pod-setting, 1939. Solid lines are the calculated best-fitting lines for temperatures of 70°, 80° and 90°F. indicated by solid circles.

A summary of the mean pod-setting for the 1939 tests appears in Table XVII.

TABLE XVII

Summary of pod-setting means, in degrees, for the different temperatures, and the corresponding percentage values (1939)

	Temperatures (°F.)						S.E.	Difference for significance
	90	90-80	80	90-70	80-70	70		
Degrees	18.94	25.29	33.06	36.95	42.95	44.38	±2.084	6.07
Percentage	10.5	18.2	29.8	36.1	46.4	48.9		

With the exception of pod-setting at 80°F., the results for the six plants tested in 1939 correspond to the results for the three plants tested in 1937. A temperature of 90°F. was found to be detrimental to pod-setting under the conditions of the experiment. The 1939 results indicate that 80°F., while not as injurious as 90°F., resulted in a poorer pod-set than did a temperature of 70°F. The pod-setting at temperature changes was somewhat erratic, but in general, quite similar to pod-setting at the corresponding constant temperature. For example, the pod-setting at 80-70°F. was approximately the same as that at 70°F.

A summary of the seed counts made on pods from the 1939 tests is to be found in Table XVIII. An examination of these data reveals that, for plants in which a sufficient number of pods were used, embryo abortion seems to have occurred more frequently at the higher temperatures.

Figures 18 to 21 illustrate the size attained by seeds at different temperatures 12 to 14 days after flowers were tripped. In general, there is little difference in size between seeds formed at 90° and 80°F. Seeds developed at 70°F. in the same time are much smaller and lighter in color.

Seed weight data appear in Table XIX. Even though the seeds developed at 90° and 80°F. differed little in size, their weights differed considerably. Seed development is much more rapid at the higher temperatures, the proportional weight of twelve-day old seeds developed at 90°, 80° and 70°F. being approximately 6:4:1.

The results for tests made to compare pod-setting on plants and cuttings are given in Table XX. The various tests were made on plants growing in the field, except on March 31, when a plant in the greenhouse was used.

TABLE XVIII

Temperature effect on seed-setting, 1939

Plant Number	Temperature °F.	Pod-setting (%)	Number of pods	Number of seeds			Seeds per pod		Seeds per flower	
				Normal	Aborted	Total	Total	Normal	Total	Normal
I.28.18 (14-38)	90	2.38	--	--	--	--	--	--	--	--
	90-80	10.14	13	26	15	41	3.15	2.00	0.32	0.20
	80	31.17	--	--	--	--	--	--	--	--
	90-70	31.11	60	144	1	145	2.42	2.40	0.75	0.75
	80-70	41.18	71	188	3	191	2.70	2.65	1.11	1.10
	70	59.83	139	342	3	345	2.48	2.46	1.48	1.47
S_1.31.1 (23-4)	90	12.02	20	30	3	33	1.65	1.50	0.20	0.18
	90-80	10.28	6	12	0	12	2.00	2.00	0.21	0.21
	80	35.10	80	121	28	149	1.86	1.51	0.65	0.53
	90-70	24.88	52	69	2	71	1.37	1.33	0.34	0.33
	80-70	46.70	92	135	25	160	1.74	1.47	0.81	0.69
	70	41.44	72	126	4	130	1.81	1.75	0.75	0.73
I.31.9 (21-35)	90	12.13	28	24	30	54	1.93	0.86	0.23	0.10
	90-80	31.00	84	114	48	162	1.93	1.36	0.60	0.42
	80	16.99	34	59	17	76	2.24	1.74	0.38	0.29
	90-70	35.96	73	140	5	145	1.99	1.92	0.71	0.69
	80-70	36.93	88	159	12	171	1.94	1.81	0.72	0.67
	70	36.26	95	149	8	157	1.65	1.57	0.60	0.57

TABLE XVIII (Continued)

Plant Number	Temperature °F.	Pod-setting (%)	Number of pods	Number of seeds			Seeds per pod		Seeds per flower	
				Normal	Aborted	Total	Total	Normal	Total	Normal
S_2.32.7 (10-34)	90	3.85	4	6	1	7	1.75	1.50	0.07	0.06
	90-80	3.39	3	4	2	6	2.00	1.33	0.07	0.05
	80	17.80	12	14	7	21	1.75	1.17	0.31	0.21
	90-70	21.50	21	31	6	37	1.76	1.48	0.38	0.32
	80-70	31.86	32	49	9	58	1.81	1.53	0.58	0.49
	70	-	--	--	-	--	--	--	--	--
I.31.9 (21-23)	90	39.55	57	52	159	211	3.70	0.91	1.46	0.36
	90-80	44.32	76	105	195	300	3.95	1.38	1.75	0.61
	80	53.64	53	63	131	194	3.66	1.19	1.96	0.64
	90-70	61.22	90	274	49	323	3.59	3.04	2.20	1.86
	80-70	70.25	108	333	47	380	3.52	3.08	2.47	2.17
	70	64.00	93	228	51	279	3.00	2.45	1.92	1.57
S_2.32.26 (34-5)	90	5.56	3	5	3	8	2.67	1.67	0.15	0.09
	90-80	--	--	--	-	--	--	--	--	--
	80	27.63	19	14	34	48	2.53	0.74	0.70	0.20
	90-70	44.62	57	112	17	129	2.26	1.96	1.01	0.88
	80-70	51.54	64	114	43	157	2.45	1.78	1.26	0.92
	70	56.82	75	154	44	198	2.64	2.05	1.50	1.17

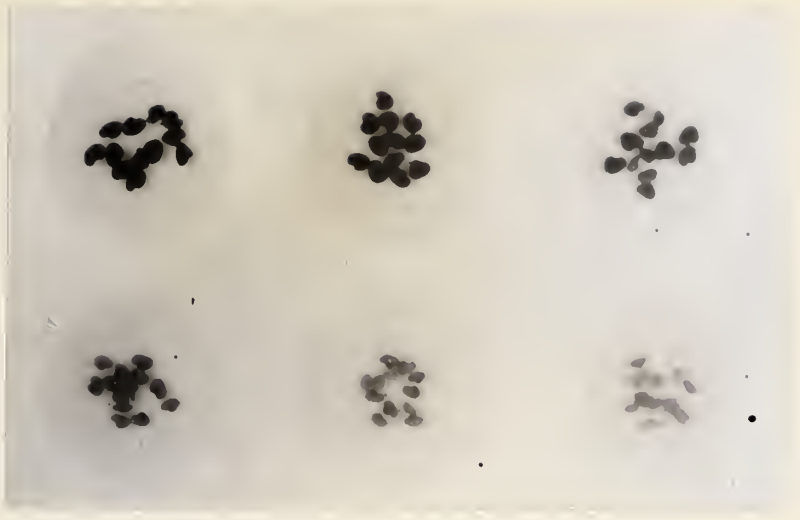

Figure 18

Fourteen-day old alfalfa seeds developed at different temperatures (Plant S_1.31.1 (23-4))

Top row - 90°F.; 90-80°F.; 80°F.
Bottom row - 90-70°F.; 80-70°F.; 70°F.

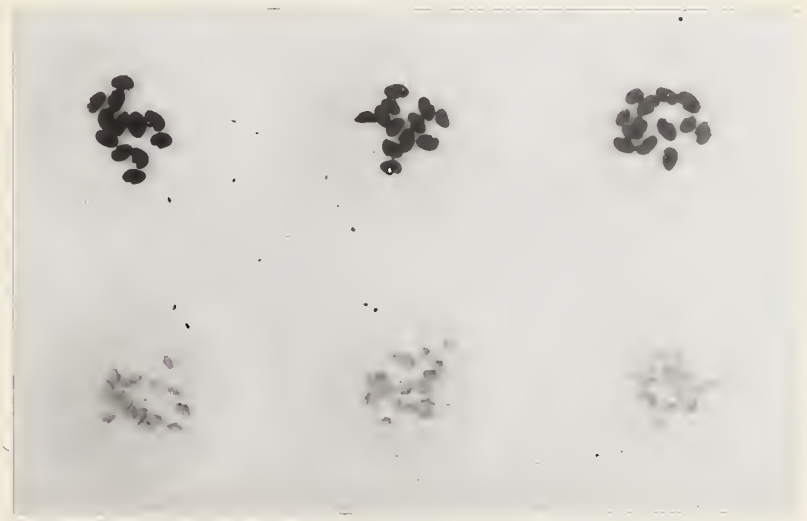

Figure 19

Twelve-day old alfalfa seeds developed at different temperatures (Plant I.31.9 (21-35))

Top row - 90°F.; 90-80°F.; 80°F.
Bottom row - 90-70°F.; 80-70°F.; 70°F.

Figure 20

Twelve-day old alfalfa seeds developed at different temperatures (Plant S_2.32.7 (10-34))

Top row - 90°F.; 90-80°F.; 80°F.
Bottom row - 90-70°F.; 80-70°F.; mature alfalfa seed*

* Not from plant S_2.32.7 (10-34)

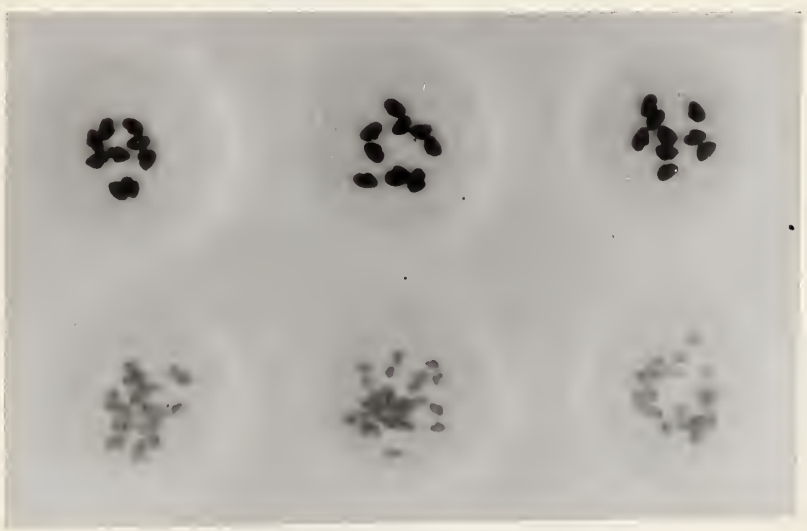

Figure 21

Twelve-day old alfalfa seeds developed at different temperatures (Plant I.31.9 (21-23))

Top row - 90°F.; 90-80°F.; 80°F.
Bottom row - 90-70°F.; 80-70°F.; 70°F.

TABLE XIX

Juvenile seed weight as affected by temperature

Temperature °F.	Plants								
	I.28.18 (14-38)*			S_1.31.1 (23-4)			I.31.9 (21-35)		
	Number of seeds weighed	Weight per 1000 seeds gm.	Proportional weight of seeds**	Number of seeds weighed	Weight per 1000 seeds gm.	Proportional weight of seeds	Number of seeds weighed	Weight per 1000 seeds gm.	Proportional weight of seeds
90	--	--	--	30	0.6967	8.69	19	0.4105	6.52
90-80	24	0.3458	6.23	12	0.4667	5.82	106	0.2406	3.82
80	--	--	--	119	0.3849	4.80	51	0.2431	3.86
90-70	141	0.0894	1.61	69	0.1551	1.93	106	0.0726	1.15
80-70	181	0.0696	1.25	134	0.1022	1.27	152	0.0658	1.04
70	290	0.0555	1.00	121	0.0802	1.00	138	0.0630	1.00
	S_2.32.7 (10-34)			I.31.9 (21-23)					
90	6	0.4333	--	34	0.2706	5.92			
90-80	4	0.3750	--	98	0.1959	4.29			
80	13	0.2462	--	56	0.1804	3.95			
90-70	23	0.0783	--	231	0.0580	1.27			
80-70	42	0.0571	--	281	0.0509	1.11			
70	--	--	--	184	0.0457	1.00			

* The seed from plants I.28.18 (14-38) and S_1.31.1 (23-4) were from two-week old pods, while the seed from the other three plants were from 12-day old pods.

** For each plant, the weight of 1000 seeds produced at 70°F. was taken as unity.

TABLE XX

Pod-setting compared on plants and stem cuttings

Plant Number	Date	Location	Duration of test (days)	Experimental condition	Number of flowers tripped	Number of pods set	Pod-setting (%)
I.28.18 (14-38)	5/7/39	Screenhouse	12	Plant	410	350	85.37
				Cuttings	410	350	85.37
I.31.9 (21-35)	5/7/39	Outside screenhouse	11	Plant	382	287	75.13
				Cuttings	390	163	41.79
I.31.9 (21-35)	18/7/39	Screenhouse	11	Plant	237	60	25.32
				Cuttings	220	50	22.73
I.31.9 (21-35)	20/7/39	Screenhouse	9	Plant	203	50	24.63
				Cuttings	239	100	41.84
S_2.32.29 (40-10)	20/7/39	Screenhouse	9	Plant	198	47	23.74
				Cuttings	201	30	14.93
I.31.9 (21-23)	31/3/39	Greenhouse	13	Plant	104	88	84.62
				Cuttings	163	146	89.57

In three of the six tests, pods were set equally well on both the plant and cuttings. For plant I.31.9 (21-35), pod-setting was better on the plant at one date, and at another date it was better on cuttings. The duration of these two tests was 11 and 9 days, respectively, but this fact alone is hardly sufficient to explain the difference in results.

Seed counts were made on pods formed in the test of March 31, and the results appear in Table XXI.

TABLE XXI

Seed-setting on plant and cuttings
(Plant I.31.9 (21-23))

	Pod-setting (%)	Number of pods	Number of seeds			Seeds per pod		Seeds per flower	
			Normal	Aborted	Total	Total	Normal	Total	Normal
On plant	84.62	101	451	5	456	4.51	4.47	3.82	3.78
On cuttings	89.57	144	526	13	539	3.74	3.65	3.35	3.27

The total number of seeds formed per pod was greater on the plant than on cuttings, but the amount of embryo abortion did not differ greatly.

Discussion

In agreement with Alter (2), a temperature of 100°F. was found to be too high for pod-setting under the experimental conditions used. Pod-setting results obtained for plant S_2.32.7 (10-34) might indicate that a temperature of 70°F. is too low for optimum pod-setting of certain plant genotypes. The differential reaction of plants to temperature seems evident when the 1937 results are considered (Table XII, Figure 16). Plant S_1.32.32 (47-5) set pods equally well at all of the temperatures used; plant S_2.32.7 (10-34) gave the best pod-setting at 80°F.; and for the other plants a temperature of 70°F. was best.

Seed-setting, as measured by the total number of seeds per pod, did not seem to be influenced greatly by different temperatures. The results indicate, however, that temperature does have some effect on the number of normal seeds produced in each pod. These results may be misleading, and another explanation is possible. As is shown by the illustrations (Figures 18-21) and seed weight data (Table XIX), the development of seeds is much more rapid at 90° than at 70°F. Abortion of the seed may occur at any stage of

development, from the time of fertilization to maturity (Cooper, Brink and Albrecht (17)). It is quite possible that, had the seeds produced at 70°F. been allowed to grow to the same size as was attained by seeds at 90°F. in 12 to 14 days, the abortion would have been greater, perhaps equal to that occurring at 90°F.

The results, from tests made to compare pod-setting on the plants themselves with that on stem cuttings, would lead to the belief that cuttings might well be used for testing the self-fertility of alfalfa plants. However, more work needs to be done before arriving at any definite conclusions.

If stem cuttings are to be used for estimating the self-fertility of individual alfalfa plants for breeding purposes, it would seem reasonable to base selection on the number of seeds per flower, rather than on either the pod-setting percentage or the number of seeds per pod. The relative amount of embryo abortion should also be considered.

GENERAL CONCLUSIONS

Investigations relative to some of the physiological factors which may affect the fertility of alfalfa have been reported. Variations in pod-setting of the individual plant throughout the season, as demonstrated by Torsell (32), Bolton (5) and others, cannot be due to pollen viability.

Temperature has a decided effect on pollen tube growth, and this may have some bearing on the results obtained for pod- and seed-setting at different temperatures.

Different concentrations of both oxygen and carbon dioxide affect pollen germination. The significance of this in relation to seed-setting is problematical. It has been suggested that the atmosphere inside the keel of untripped flowers may contain too high a concentration of carbon dioxide to permit pollen germination. The moisture relationships of the pollen, as discussed by Martin (23) must also be considered, along with the proportion of gas components of the atmosphere within the keel.

Pod- and seed-setting are influenced by temperature, and seed development is more rapid at higher temperatures. The amount of embryo abortion may also be affected by temperature. If the theory formulated by Brink and Cooper (10) is correct, it is quite probable that different temperatures would influence the amount of embryo abortion.

Preliminary tests indicate that the use of stem cuttings, for estimations of self-fertility of alfalfa plants, may be feasible. If such a system were to be used, the number of normal seeds produced per flower should be considered as the criterion of potential self-fertility.

SUMMARY

1. Viability of the pollen produced by individual plants does not vary significantly throughout the flowering season. Therefore, the seasonal variations in pod-setting cannot be due to changes in pollen viability.

2. A linear relationship was found to exist between pollen tube growth and temperature, tube length increasing as the temperature increased from 70° to 100°F.

3. The necessity of oxygen for germination of alfalfa pollen has been demonstrated. When an atmosphere contains over 40 percent oxygen, the percentage pollen germination is somewhat depressed.

4. The percentage germination of pollen on an agar-sugar medium decreased consistently as the carbon dioxide content of the atmosphere increased, till at a concentration of 40 percent no pollen germination occurred. It is suggested that the atmosphere inside the keel of untripped flowers may possess a high concentration of carbon dioxide which, in combination with a comparatively low humidity, may be sufficient to prevent pollen germination.

5. The results obtained from tests made on stem cuttings in tap water indicate that temperature affects pod- and seed-setting. A temperature of 100°F. was too high for pod-formation in a two-week test period. At 90°, 80° and 70°F. pod-setting, in general, increased with a decline in temperature. Differential reaction of plants to temperature in relation to pod-setting has also been indicated.

6. The rate of seed development is greatly influenced by temperature. The proportional weights of twelve-day old seeds developed at temperatures of 90°, 80° and 70°F. are approximately 6:4:1.

7. The amount of embryo abortion appears to be greater at 90°F. than at 70°F. Whether the increase in embryo abortion is actually due to temperature or to the rate of development is not clear, but temperature may be the determining factor if the theory of Brink and Cooper (10) is considered.

8. The use of stem cuttings for estimating the self-fertility of alfalfa plants is discussed. It is suggested that, for comparisons, the number of seeds per flower should be used instead of either the percentage of pod-setting or the number of seeds per pod.

ACKNOWLEDGMENTS

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